Reference: *Biol. Bull.* **197:** 104–111. (August 1999)

The Velar Ciliature in the Brooded Larva of the Chilean Oyster *Ostrea chilensis* (Philippi, 1845)

O. R. CHAPARRO[1], R. J. THOMPSON[2,*], AND C. J. EMERSON[3]

[1] *Instituto de Biología Marina, Universidad Austral de Chile, Casilla 567, Valdivia, Chile;*
[2] *Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1C 5S7; and* [3] *Biology Department, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9*

Abstract. The Chilean oyster (*Ostrea chilensis*) broods its offspring almost to the settlement stage (about 8 weeks). Larvae are maintained inside the infrabranchial chamber of the female. Samples from all embryo and larval developmental stages were obtained from mantle cavities of brooding females and analyzed by scanning electron microscopy, with particular attention to the velar structures.

All embryos and the earliest veliger stages of *O. chilensis* are devoid of cilia. Cilia first appear when shell length reaches 290–300 μm, and the first cilia to grow on the velum form the outer preoral cilia. In larvae 340 μm long, all the ciliary rings on the velum can be distinguished. These are the apical cilia (AC), inner preoral cilia (IPC), outer preoral cilia (OPC), and adoral cilia (AOC). The absence of the apical tuft in both *O. chilensis* and the closely related species *O. edulis* represents an adaptation to brooding by the embryos and larvae, but the lack of the postoral cilia (POC) in *O. chilensis* and the lack of cilia in the embryonic and early veliger stages are associated with an extreme brooding condition in this species.

Introduction

Most bivalve molluscs exhibit external fertilization of the gametes followed by the development of pelagic larvae. In some species, however, there is a totally benthic or brooded larval development, and in other cases a period of brooding is followed by a pelagic phase (Pechenik, 1979, 1986). In brooding species the eggs, embryos, and larvae are retained in the interlamellar spaces of both demibranchs or of the inner or outer demibranchs only; alternatively, they may be confined to brood sacs, marsupia, mucous masses, capsules, or other specialized structures (Ockelmann, 1964; Solís, 1967; Franz, 1973; Mackie *et al.*, 1974; Heard, 1977; Mackie, 1984; Tankersley and Dimock, 1992, 1993; Gallardo, 1993).

Brooding is a characteristic common to all members of the subfamily Ostreinae (Harry, 1985). All species of the genus *Ostrea* brood their embryos in the infrabranchial chamber (Millar and Hollis, 1963; Galtsoff, 1964; Chanley and Dinamani, 1980; Harry, 1985; Cranfield and Michael, 1989). Brooding in oysters can be very short, as in *O. puelchana* (3 days, Morriconi and Calvo, 1980; 3 to 9 days, Fernandez Castro and Le Pennec, 1988), or very long, as in *O. chilensis* (6 to 12 weeks; Toro and Chaparro, 1990). In addition to having the longest period of brooding, the Chilean oyster produces the fewest eggs (3500 to 152,000) of any *Ostrea* species, with the largest egg diameter (approximately 250 μm), the largest pediveliger at the time of release (approximately 450 μm), and the shortest pelagic phase (minutes to 24 hours) (review by Toro and Chaparro, 1990).

Pelagic larvae possess structures specialized for swimming and feeding, but it is unlikely that brooded larvae have the same requirements, especially if the brooding period is long, as in the Chilean oyster (8 weeks). In many species, brooded larvae do not ingest particles, often because the brooding female provides nutrition through the biochemical reserves in large eggs, as in *O. chilensis*, or through body fluids or nurse eggs. Strathmann (1978) has described the adaptations of some nonfeeding brooded larvae. In other cases, the female concentrates phytoplankton and other suspended material from the external environment for the use

Received 22 May 1998; accepted 24 May 1999.

* To whom correspondence should be addressed. E-mail: thompson@morgan.ucs.mun.ca

of the larvae, as suggested by Buroker (1985) for *Ostrea* spp. and by Mackie (1979) for freshwater bivalves (Pisidiidae), and as demonstrated in *O. chilensis* by Chaparro *et al.* (1993). The present paper examines the velar ciliature of the larva of *O. chilensis*, an extreme case in which the larva is brooded for almost the entire developmental period, and compares the ciliature morphologically with that of the planktotrophic larvae of related species, particularly other ostreids.

Materials and Methods

Samples of oysters (*Ostrea chilensis*) were obtained at intervals throughout the brooding period (October to January) during 1992, 1993, and 1994 from a natural bank in the Quempillén estuary in the northern part of Chiloé Island (41°52'S; 73°46'W), in the south of Chile. On each sampling date, several female oysters were opened and their embryos or larvae removed. In this way, all larval development stages were sampled. Larvae were prepared for scanning electron microscopy (SEM) following Hadfield and Iaea (1989).

Larvae were anesthetized for about 10 min in a $MgCl_2$ solution isotonic with seawater, then fixed for 1 h in ice-cold 3% glutaraldehyde in 0.2 *M* sodium cacodylate buffer, pH 7.4. Fixed samples were rinsed in the buffer solution twice and post-fixed for 1 h in ice-cold 1% OsO_4 in 0.2 *M* sodium cacodylate, pH 7.4. The specimens were then rinsed two or three times with buffer solution and then once with distilled water before being dehydrated in a graded series of ethanol (Cragg, 1985).

For SEM, dehydrated specimens were critical-point dried from liquid carbon dioxide in a Polaron E3000 drying apparatus. Dried larvae were attached to aluminum viewing stubs with double-sided tape and then coated with gold in an Edwards S150A sputter-coater. When necessary, larval shells were broken with a fine needle to expose the internal structures (Cragg, 1985, 1989). Coated samples were viewed in a Hitachi S570 scanning electron microscope operated at an accelerating voltage of 20 kV. Micrographs were recorded on Polaroid Type 665 positive/negative film, and stereopairs taken with a 10° tilt angle difference.

Each brood of larvae was processed separately. Although all larvae from a given brood were at the same developmental stage, as an additional precaution at least 30–50 larvae from each brood were observed by SEM before micrographs were taken, to ensure that the structures observed were common to all of them. Measurements based on SEM are approximate, owing to foreshortening effects related to the depth of field, the tilt angle, and the curvature of the sample.

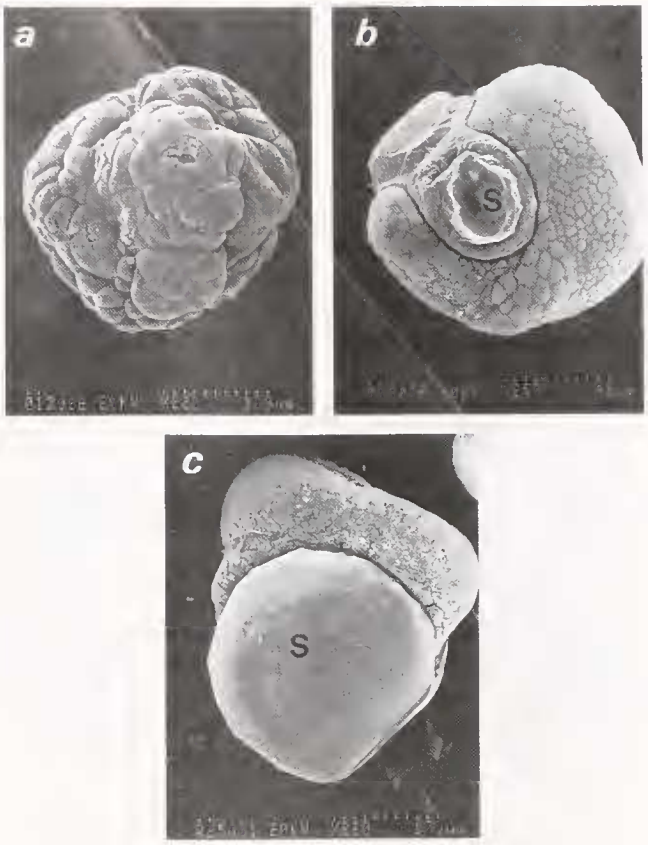

Figure 1. Early development stages in *Ostrea chilensis*. (a) Gastrula; scale bar 109 μm. (b) Dorsolateral view of late trochophore; scale bar 96 μm. (c) Lateral view of early veliger; scale bar 109 μm. In all cases, embryos or larvae are devoid of cilia.

Results

Early development stages

The earliest development stages are naked, with no cilia (Fig. 1). Only when the embryo reaches the earliest veliger stage, with a shell length of about 290 μm, is the first ciliary growth observed. Cilia appear on the upper part of the velum, and owing to their location and arrangement on the velum, they are presumed to form the future ring of outer preoral cilia (OPC) (Fig. 2a, b). These cilia are about 11–14 μm long. At this stage they are separate, with a tendency to join each other in the middle basal part of the cilia. At the same time, a group of short cilia develops in the mouth region and begins to cover the food groove. These cilia are shorter than those in the putative OPC ring, and are randomly distributed (Fig. 2c).

After 25–30 days of brooding (shell length 315–320 μm), a clear pattern of single or compound cilia has emerged which persists for the remainder of the larval phase. The ciliary belts composing the velum are shown schematically in Figure 3. Larvae exhibit a very well synchronized ciliary

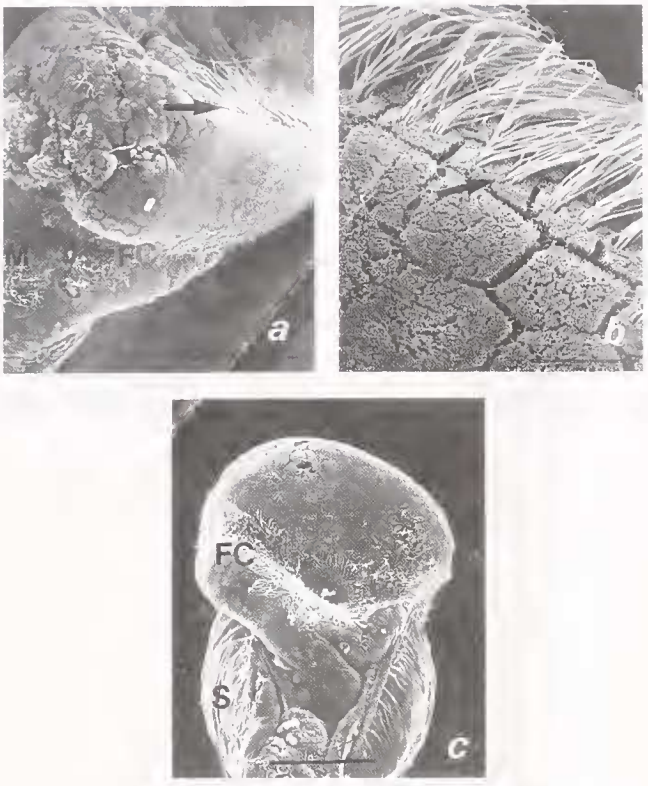

Figure 2. In a larva of *Ostrea chilensis* with a shell length of about 300 μm, the cilia first appear on top of the velum, as well as in the food groove and mouth region. (a, b) Superior-lateral view of the cilia that will form the OPC band; scale bars 60 μm and 8 μm respectively; arrows indicate future OPC. (c) Short cilia covering the adoral food groove (FC) and the mouth region (ventral aspect); scale bar 80 μm.

growth pattern, with all larvae from the same brood being at the same developmental stage.

Distribution of cilia on the velum

The ciliature of a larva of shell length 400 μm is shown in Figure 4A. A concavity about 70–80 μm in diameter is visible in the most central and apical sector of the velum (Fig. 4B). Located in its base is a group of small cilia, the apical cilia (AC), which are randomly distributed and are not organized into the apical tuft characteristic of planktonic veligers, but lie in the same position. Surrounding this depression is a bare region about 60 μm wide, delimited by a single belt of cilia constituting the inner preoral ciliary (IPC) ring.

Inner preoral cilia

The IPC form a ring of single cilia (Fig. 4C), each one about 15–20 μm long. Outside the IPC ring is a naked area, 9–10 μm wide, which is surrounded by a large ring of OPC.

Outer preoral cilia

The OPC form a band, about 10–15 μm wide, of two rows of cirri, or composite cilia (Waller, 1981), which are oriented radially from the center of the velum (Fig. 4D, E). This is the dominant ring in the velum because of its width and the size and complexity of its cirri. Each cirrus is roughly 80 μm long and is composed of 50–100 cilia,

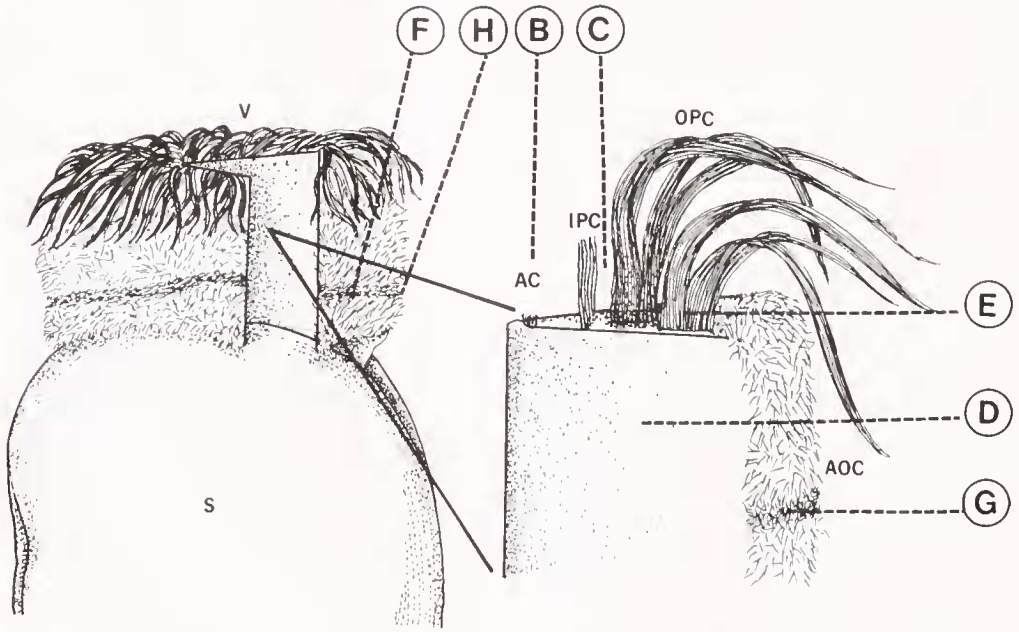

Figure 3. Schematic representation of the velum in a late pediveliger of *Ostrea chilensis* (>400 μm shell length) in lateral view. Ciliary bands are identified in an enlarged section of the velum. AC: apical cilia; AOC: adoral cilia; IPC: inner preoral cilia; OPC: outer preoral cilia; S: shell; V: velum. Circled letters refer to the scanning electron micrographs in Figure 4.

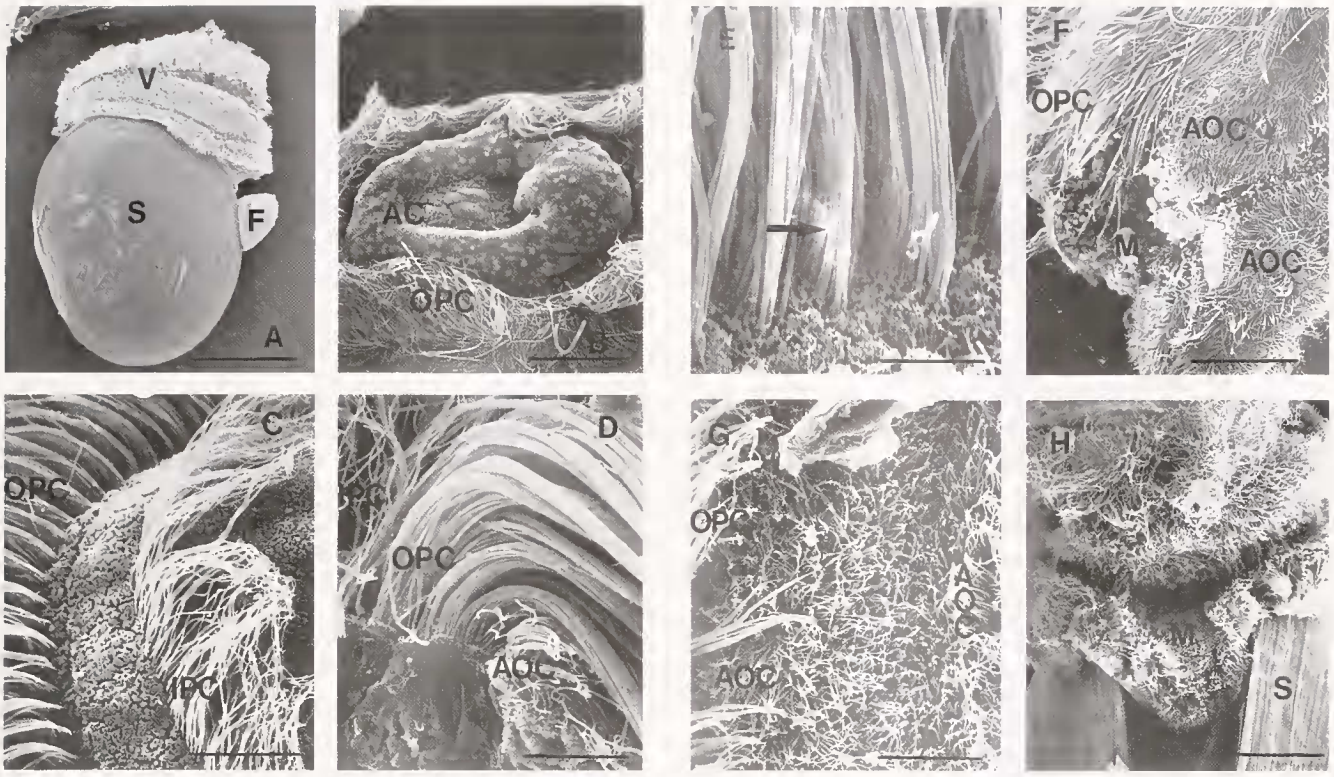

Figure 4. Ciliature of the late pediveliger of *Ostrea chilensis*. (A) Lateral view; scale bar 160 μm. (B) Superior view of the velum showing the AC band in the center; scale bar 60 μm. (C) Detail of the tPC band (superior aspect); scale bar 10.9 μm. (D) Transverse section of the velum showing the principal ciliary bands; scale bar 9.6 μm. (E) Detail of the OPC band; scale bar 3.0 μm. Arrow indicates a row of cirri. (F) Lateral view of the mouth area, showing OPC and AOC bands; scale bar 40 μm. (G) Food groove; scale bar 22 μm. (H) Ventral (= frontal) view of the mouth region; scale bar 48 μm. F = foot; for other abbreviations see legend to Figure 3.

which are in contact with each other for almost all their length. The width of the cirrus is about 1 μm, and each one is separated from its neighbor by a nonciliated space of about 1.5–2 μm.

Adoral cilia

The remainder of the velum (adoral area), from the OPC to the dorsal margin of the shell, is covered by a carpet of individual, short cilia (8–12 μm) known as the adoral cilia (AOC), which also cover the crest and floor of the food groove (the sides are not visible) and the external part of the mouth. No differentiation in the form of the AOC is apparent, although in some areas there are a few cilia of different length, and in the floor of the food groove the cilia are shorter than the rest of the AOC (Fig. 4F, G). Short cilia can also be seen on the base of the external part of the mouth (Fig. 4H). There is no clearly identifiable postoral ciliary band of the type identified in larvae of *Ostrea edulis* by Waller (1981). Below the food groove and close to the mouth lie several lobes, which resemble the tumescent cells identified by Waller (1981) in *O. edulis*.

Discussion

The inclusion of a pelagic larva in the life cycle of most marine bivalve molluscs is accompanied by anatomical adaptations, especially those associated with the velum, and is characterized by rapid development to the prodissoconch stage. These features allow the larva to survive for long periods in the water column, and the velum is an adaptation for planktonic life (Cragg, 1989). Many papers on the early development of bivalves have shown the presence of cilia on some parts of the embryo or larva, or covering the entire surface (Allen, 1961; Carriker, 1961; Bayne, 1976; Amor, 1981; Fitt *et al.*, 1984; Gustafson and Lutz, 1991). In most cases the cilia appear at the earliest stages of embryonic development (Gallardo, 1989). The few studies that have been undertaken on brooded embryos or larvae have shown that cilia first appear at later stages of development: *Ostrea lurida*, trochophore stage (Hopkins, 1936); *O. edulis*, trochophore stage (Horst 1883–1884, in Waller, 1981). However, the present study shows that in the Chilean oyster all embryonic stages, together with the trochophore and early veliger, are totally devoid of cilia. This is one of the few

cases known in which cilia are totally absent at such a late stage of development in a bivalve. From the illustrations in Beauchamp (1986), it can be inferred that advanced, shelled veligers of the brooding clam *Lasaea subviridis* are also naked. This species exhibits direct development, and no pelagic phase has been detected (Beauchamp, 1986), an observation that would explain the absence of cilia during the larval stages. Oldfield (1964) demonstrated that the area normally occupied by the ciliated velum is replaced in *L. rubra* by an unciliated structure she termed the "cephalic mass," implying a specialization for brooding more extreme than is seen in the Chilean oyster. Thus loss or modification of the velar ciliature represents one of the most important and visible adaptations for brooding in a bivalve mollusc.

Cilia begin to develop when the shell length of the Chilean oyster larva is about 290 μm. By the time the larva reaches about 300 μm, the cilia have developed completely and remain throughout the rest of the larval phase. The present study supports the contention of Winter *et al.* (1984) and Toro and Chaparro (1990) that the brooding period in *O. chilensis* is the longest of any *Ostrea* species for which information is available. There is little necessity for swimming because the larva spends only a few hours in the water column and is competent to settle immediately after being released (Solís, 1973; Cranfield, 1979). According to Millar and Hollis (1963), the shortened pelagic phase in the larva of *O. chilensis* is an adaptation for survival in a habitat characterized by strong currents.

The absence of cilia during much of larval life may be considered an adaptation to brooding. The encapsulated embryos of many marine invertebrate species lacking pelagic larvae bear structures that appear to be functionally significant only in free-swimming larval stages. In some encapsulated embryos, those cilia normally associated with swimming in pelagic larvae serve to rotate the embryo within its capsule, presumably aiding the larva in the ingestion of fluid albumen and in gas exchange (Fretter and Graham, 1962). Hadfield and Iaea (1989) reported an extreme case in which encapsulated larvae of the gastropod *Petaloconchus montereyensis* showed an absence or reduction of some velar structures that are very important in closely related species with a pelagic larva. On the other hand, in another encapsulated gastropod (*Turritella communis*), cilia develop in the very earliest embryonic stages, even though these cilia are not required for swimming (Kennedy and Keegan, 1992). The velar lobes of many encapsulated gastropod veligers are known to participate in the breakdown and ingestion of nurse eggs (Fioroni, 1966), and in others the velum is greatly modified for aiding in the ingestion and perhaps in the breakdown of external nurse yolk (Hadfield and Iaea, 1989).

In Chilean oyster veligers, ciliary development is well-synchronized within a brood. Immediately after the cilia have completely developed, the larvae start to ingest particles. Thus the cilia are required for feeding more than for swimming, although data from endoscopy show that the larvae are not completely immobile, but exhibit a specific circulation pattern inside the mantle cavity of the female (Chaparro *et al.*, 1993). The larval circulation is driven by water currents produced by the female rather than by the larval velum. Furthermore, although the velar cilia are very active when the female has stopped pumping, the larvae move very little, and it is not clear whether they are capable of swimming at this stage.

The composition of the ciliary bands on the velum is similar, but not identical, to that of the closely related species *Ostrea edulis*, described by Waller (1981). Both these species have a short apical ciliary (AC) ring and no apical tuft, unlike the pelagic larvae of many other bivalves (Allen, 1961; Carriker, 1961; Ansell, 1962; Gruffydd and Beaumont, 1972; Bayne, 1976; Boyle and Turner, 1976; Chanley and Chanley, 1980; Amor, 1981). The reduced development of the AC ring in the Chilean oyster larva may be explained by a reduced sensory function during the brooding phase, and also by the short larval pelagic phase. A sensory function has been proposed for the AC because they are very short, appear to be unsuitable for locomotion and food-gathering, and are underlain by the cerebral ganglion (Hickman and Gruffydd, 1971). According to Hodgson and Burke (1988), the apical tuft remains in the larva of *Chlamys hastata* until the earliest veliger stage, but in the Chilean oyster larva the AC never develop into a tuft.

A ring composed of single cilia (inner preoral cilia, IPC) has been identified between the AC and the OPC in the Chilean oyster veliger. A similar structure has also been described by Waller (1981) for *O. edulis*, by Hodgson and Burke (1988) for *Chlamys hastata*, and by Elston (1980) for *Crassostrea virginica*. No clear function has been identified for this band (Waller, 1981). Although the IPC ring is situated in such a position that it could entrain food particles, it lies far from the mouth and the AOC and is separated from the nearest ciliated pathway to the mouth by a non-ciliated zone. Waller (1981) therefore concluded that the IPC probably do not play a role in food capture and are more likely to function as an upcurrent tactile receptor.

The outer preoral cilia (OPC) form the most prominent ciliated ring in the velum of *O. chilensis*, as they do in several other bivalve larvae including *O. edulis* (Waller, 1981), *Chlamys hastata* (Hodgson and Burke, 1988), and *Crassostrea virginica* (Elston, 1980). In some species the ring is composed of a single row of cilia in the early larval stages (*e.g.*, the D-stage veliger of *C. hastata*), but more developed larvae of *C. hastata* have two rows (Hodgson and Burke, 1988), as do all stages of the veliger in *O. edulis* (Waller, 1981). The OPC ring is believed to function in locomotion and feeding (Strathmann *et al.*, 1972; Strathmann and Leise, 1979; Waller, 1981), and it is normally the most prominent ciliated structure on the velum (Waller,

1981). Furthermore, Bayne (1971) showed that long cilia, presumably the OPC, on the velum of the pediveliger of the mussel *Mytilus edulis* provide the main force for swimming and also create the feeding currents.

In *O. chilensis*, a wide band of short adoral cilia (AOC) covers an area of the velum limited in the uppermost part by the OPC ring and in the lowest part by the shell. In the middle of this band lies the food groove. The ciliature is uniform throughout the band, although the cilia in the floor of the food groove are a little shorter. The cilia of the upper and lower regions of the AOC band are in close contact with the groove. These cilia are probably responsible for transferring particles caught by the other ciliary bands to the food groove. In many specimens of *O. chilensis* veligers, pieces of mucous strings or globules could be distinguished in the food groove, presumably moving towards the mouth. It may be significant that some descriptions of feeding in lamellibranch veligers (*e.g.*, Yonge, 1926) refer to a mucous string carrying food particles to the mouth, since the efficiency of a system that lacks postoral cilia may be improved by the presence of mucus (Bayne, 1976). Waller (1981) indicated that the AOC are in close contact with the compound cilia of the OPC when the latter are at the bottom of their effective beat; this is probably the mechanism by which the OPC transfer the entrained particles to the AOC band to be moved to the mouth.

The velar bands are almost identical in *O. chilensis* and *O. edulis*, the principal difference being that in the latter the postoral cilia (POC) represent a single cirral ring located at the base of the velum, in contact with the shell edge (Erdmann, 1935; Waller, 1981), whereas the POC were not visible in this study on the Chilean oyster and are probably not present. On a cautionary note, however, Strathmann *et al.* (1972) pointed out that some authors have not mentioned the POC when describing mollusc larvae that feed and have a well-developed preoral band. These authors suggested that in many cases this may have been an oversight, or a result of confusing the shorter cilia of the postoral band with the cilia of the food groove. However, owing to the central position of the food groove on the velum of the Chilean oyster larva, it would be easy to distinguish between food groove cilia and POC, were the latter present.

Waller (1981) associated the POC band in *O. edulis* with both swimming and particle capture. The latter has been described by Strathmann *et al.* (1972), who showed that many marine invertebrate larvae can continue swimming without feeding, presumably by stopping the beat of the POC. This band appears to be very important in filter-feeding, especially in the larvae of taxa which employ the opposing band mechanism proposed by Strathmann *et al.* (1972) and Strathmann (1978)—bivalves, annelids, echiurids, sipunculids, and entoprocts. In this system both preoral and postoral rings are essential for filter-feeding by the larva (Strathmann *et al.*, 1972), and provide an efficient mechanism to capture particles. In this context, the POC ring identified by Waller (1981) in *O. edulis* may serve to catch particles passing the tips of the cirri of the preoral band, and may also play a more direct role in particle retention and rejection (Strathmann *et al.*, 1972).

The food collected by the cirri is moved to the adoral ring and then to the mouth, where oral compound cilia are present. These may function in selecting or rejecting food particles before they enter the mouth (Hodgson and Burke, 1988).

Whatever the characteristics of the POC band, Hodgson and Burke (1988) have suggested that it may play a role in particle capture, although Strathmann (1987) has shown that the larvae of other invertebrate taxa catch particles by using only one cirral ring. In the Chilean oyster, the particle-catching mechanism was not identified in the present study, but the apparent absence of the second (POC) ring may imply that only one ciliary band is involved, probably because the brooded larva does not need to concentrate particles since the brooding female is performing this function. Furthermore, endoscopic observations have shown the larval velum to be in close contact with mucous strings from the food grooves on the gills of the female (Chaparro *et al.*, 1993). The resolution of the endoscope was insufficient to determine whether larvae were ingesting the mucous string, but the presence of larvae in the food groove, their orientation, and their behavior suggested that this was probable; furthermore, marker particles introduced into the ambient seawater were later observed in the gut of the larva. Thus the absence of the POC may represent another adaptation of the Chilean oyster larva for brooding. Hodgson and Burke (1988) identified secretory cells among the velar cilia of the pectinid *Chlamys hastata*, but such cells have not been described in other bivalve larvae, although several authors have suggested or assumed that mucus is involved in the collection of particles (Yonge, 1926; Erdmann, 1935; Strathmann *et al.*, 1972; Waller, 1981). Hodgson and Burke (1988) suggested that mucus produced by the velum serves principally to bind entrained particles into a string, which travels along the food groove, thereby ensuring the retention of the particles. Pieces of mucus were detected in the food groove of the larva of the Chilean oyster during the present study. The origin of this material could not be clearly ascertained, but two possibilities may be suggested: first, that pieces of the mucous string are taken from the food grooves of the female's gill; and second, that mucus is produced by the larva, as described by Hodgson and Burke (1988) in *C. hastata*.

The absence of the POC band is consistent with the short pelagic phase of the pediveliger in *O. chilensis* because there is no requirement to filter food particles from the water column and no necessity to swim for a long time as a dispersive strategy, since the population is confined to its own estuary. The pediveliger is competent to settle on the

first hard substrate that it encounters after release. Typical settlement distances are variously given as 10 cm (Padilla *et al.,* 1969) and 40 cm (Solís, 1973) from the female parent.

Morphological adaptations of marine invertebrate larvae have been described by Strathmann (1978). The larvae of some oligomeric taxa (*e.g.,* Bryozoa, Phoronida, Brachiopoda, Hemichordata) have a single band system and have eliminated the planktotrophic stage. These taxa have lost or modified some larval structures, such as the band of cilia that captures particles. The gut is often incomplete, and the mouth, anus, or both may be absent. In some mollusc species with nonfeeding larvae, the metatroch and food groove have been lost; in contrast, other gastropod larvae, which are also not filter-feeders, retain the opposing band system, even when the larvae are not planktotrophic (Strathmann, 1978). It is clear that many species have modified some larval structures, allowing the larva to adapt more completely to the environment in which it is developing. The Chilean oyster is one such example: the morphological modifications of the velum are essential for the adaptation by the species to brooding the larva inside the mantle cavity of the female—a very different environment from that experienced by a planktonic larva. Furthermore, in *O. chilensis* and *O. lutaria,* which have the longest incubation periods of all the ostreids, the larval shells differ structurally from those of other oysters (Chanley and Dinamani, 1980). In particular, the shells of the two larvae are equivalve and edentulous, but it is not clear whether these features represent the ancestral condition or are adaptations to brooding (Chanley and Dinamani, 1980).

Acknowledgments

A large part of the field research was carried out in the Estación Experimental de Quempillén, Ancud, Chile, and in the Instituto de Biología Marina de la Universidad Austral de Chile, to whose staff we extend our appreciation. Financial help came from operating grants to ORC by the Fondo Nacional de Investigación Científica y Tecnológica-Chile (Fondecyt 1930364 and 1980984), by the International Foundation for Sciences-Sweden (IFS A/0846), and by the Dirección de Investigación of the Universidad Austral de Chile, and from a research grant to RJT from the Natural Sciences and Engineering Research Council-Canada (NSERC). ORC's stay in Canada was made possible by fellowships from the International Development Research Centre-Canada (IDRC) and NSERC (through a Networks of Centres of Excellence programme, the Ocean Production Enhancement Network). We also thank the Canadian International Development Agency (CIDA) for support during the preparation of the manuscript.

Literature Cited

Allen, J. A. 1961. The development of *Pandora inaequivalvis* (Linné). *J. Embryol. Exp. Morphol.* **92:** 252–268.

Amor, A. 1981. Observaciones sobre el desarrollo embrionario y larval de *Mytilus platensis* D'Orbigny, del sector Bonaerense (Mollusca, Bivalvia). *Physis (Buenos Aires),* Secc. A., **39(97):** 33–39.

Ansell, A. 1962. The functional morphology of the larva, and the postlarval development of *Venus striatula* (Da Costa). *J. Mar. Biol. Assoc. U.K.* **42:** 419–443.

Bayne, B. L. 1971. Some morphological changes that occur at the metamorphosis of the larvae of *Mytilus edulis.* Pp. 259–280 in *European Marine Biology Symposium.* D. J. Crisp, ed. Cambridge University Press, London.

Bayne, B. L. 1976. The biology of mussel larvae. Pp. 81–120 in *Marine Mussels: Their Ecology and Physiology.* B. L. Bayne, ed. Cambridge University Press.

Beauchamp, K. A. 1986. Reproductive ecology of the brooding, hermaphroditic clam *Lasaea subviridis. Mar. Biol.* **93:** 225–235.

Boyle, P. J., and R. D. Turner. 1976. The larval development of the wood boring piddock *Martensia striata* (l.) (Mollusca: Bivalvia: Pholadidae). *J. Exp. Mar. Biol. Ecol.* **22:** 55–68.

Buroker, N. E. 1985. Evolutionary patterns in the family Ostreidae: larviparity *vs.* oviparity. *J. Exp. Mar. Biol. Ecol.* **90:** 233–247.

Carriker, M. R. 1961. Interrelation of functional morphology, behavior, and autecology in early stages of the bivalve *Mercenaria mercenaria. J. Elisha Mitchell Sci. Soc.* **77:** 168–242.

Chanley, P., and M. Chanley. 1980. Reproductive biology of *Arthritica crassiformis* and *A. bifurca,* two commensal bivalve molluscs (Leptonacea). *N. Z. J. Mar. Freshwater Res.* **14:** 31–43.

Chanley, P., and P. Dinamani. 1980. Comparative descriptions of some oyster larvae from New Zealand and Chile, and a description of a new genus of oyster, *Tiostrea. N. Z. J. Mar. Freshwater Res.* **14: 103–120.**

Chaparro, O. R., R. J. Thompson, and J. E. Ward. 1993. *In vivo* observations of larval brooding in the Chilean oyster, *Ostrea chilensis* Philippi, 1845. *Biol. Bull.* **185:** 365–372.

Cragg, S. M. 1985. The adductor and retractor muscles of the veliger of *Pecten maximus* (L.) (Bivalvia). *J. Molluscan Stud.* **51:** 276–283.

Cragg, S. M. 1989. The ciliated rim of the velum of larvae of *Pecten maximus* (Bivalvia: Pectinidae). *J. Molluscan Stud.* **55:** 497–508.

Cranfield, H. J. 1979. The biology of the oyster, *Ostrea lutaria* and the oyster fishery of Foveaux Strait. *Rapp. P. V. Réun. Cons. Int. Explor. Mer.* **175:** 44–49.

Cranfield, H. J., and K. P. Michael. 1989. Larvae of the incubatory oyster *Tiostrea chilensis* (Bivalvia: Ostreidae) in the plankton of central and southern New Zealand. *N. Z. J. Mar. Freshwater Res.* **23:** 51–60.

Elston, R. 1980. Functional anatomy, histology and ultrastructure of the soft tissues of the larval American oyster, *Crassostrea virginica. Proc. Natl. Shellfish Assoc.* **70:** 65–93.

Erdmann, W. 1935. Über die Entwicklung und die Anatomie der 'Ansatzreifen' Larvae von *Ostrea edulis* mit Bemerkungen uber die Lebengeschichte der Auster. *Helgol. Wiss. Meeresunters.* **19(6):** 1–25.

Fernandez Castro, N., and M. Le Pennec. 1988. Modalities of brooding and morphogenesis of larvae in *Ostrea puelchana* (D'Orbigny) under experimental rearing. *J. Mar. Biol. Assoc. U. K.* **68:** 399–407.

Fioroni, P. 1966. Zur Morphologie und Embryogenese des Darmtraktes und der Transitorischen Organe bei Prosobranchiern (Mollusca, Gastropoda). *Rev. Suisse Zool.* **73:** 621–876.

Fitt, W. K., C. R. Fisher, and R. K. Trench. 1984. Larval biology of tridacnid clams. *Aquaculture* **39:** 181–195.

Franz, D. R. 1973. Ecology and reproduction of a marine bivalve *Mysella planulata. Biol. Bull.* **144:** 93–106.

Fretter, V., and A. Graham. 1962. *British Prosobranch Molluscs.* Ray Society London. 755 pp.

Gallardo, C. S. 1989. Patrones de reproducción y ciclo vital en moluscos

marinos bénticos: Una aproximación ecológica evolutiva. *Medio Ambiente* **10(2):** 25–35.

Gallardo, C. S. 1993. Reproductive habits and life cycle of the small clam *Kingiella chilenica* (Bivalvia: Cyamiidae) in an estuarine sand flat from the South of Chile. *Mar. Biol.* **115:** 595–603.

Galtsoff, P. S. 1964. *The American Oyster* Crassostrea virginica *Gmelin. U. S. Fish Wildl. Serv. Fish. Bull.* **64:** 480 pp.

Gruffydd, Ll. D., and A. R. Beaumont. 1972. A method for rearing *Pecten maximus* larvae in the laboratory. *Mar. Biol.* **15:** 350–355.

Gustafson, R. G., and R. A. Lutz. 1991. Larval and early post-larval development of the protobranch bivalve *Solemya velum* (Mollusca: Bivalvia). *J. Mar. Biol. Assoc. U. K.* **72:** 383–402.

Hadfield, M. G., and D. K. Iaea. 1989. Velum of encapsulated veligers of *Petaloconchus* (Gastropoda), and the problem of re-evolution of planktotrophic larvae. *Bull. Mar. Sci.* **45:** 377–386.

Harry, H. W. 1985. Synopsis of the supraspecific classification of living oysters (Bivalvia: Gryphaeidae and Ostreidae). *Veliger* **28:** 121–158.

Heard, W. H. 1977. Reproduction of fingernail clams (Sphaeridae: *Sphaerium* and *Musculum*). *Malacologia* **16:** 421–455.

Hickman, R. W., and Ll. D. Gruffydd. 1971. The histology of the larva of *Ostrea edulis* during metamorphosis. Pp. 281–294 in *European Marine Biology Symposium.* D. J. Crisp, ed. Cambridge University Press, London.

Hodgson, C. A., and R. D. Burke. 1988. Development and larval morphology of the spiny scallop, *Chlamys hastata. Biol. Bull.* **174:** 303–318.

Hopkins, A. E. 1936. Ecological observations on spawning and early larval development in the Olympia oyster (*Ostrea lurida*). *Ecology* **17(4):** 551–566.

Kennedy, J. J., and B. F. Keegan. 1992. The encapsular developmental sequence of the mesogastropod *Turritella communis* (Gastropoda: Turritellidae). *J. Mar. Biol. Assoc. U. K.* **72:** 783–805.

Mackie, G. L. 1979. Dispersal mechanisms in Sphaeriidae (Mollusca: Bivalvia). *Bull. Am. Malacol. Union* **1979:** 17–21.

Mackie, G. L. 1984. Bivalves. Pp. 351–418 in *The Mollusca. Vol 7. Reproduction.* A. S. Tompa, N. H. Verdonk, and J. Van Den Biggerlaar, eds. Academic Press, Orlando, Florida.

Mackie, G. L., S. U. Qadri, and H. Clarke. 1974. Development of brood sacs in *Musculium securis* (Bivalvia: Sphaeriidae). *Nautilus* **88:** 109–111.

Millar, R. H., and P. J. Hollis. 1963. Abbreviated pelagic life of Chilean and New Zealand oysters. *Nature* **197:** 512–513.

Morriconi, E., and J. Calvo. 1980. Fertilidad y periodicidad del desove en *Ostrea puelchana. Rev. Invest. Desarrollo Pesq.* **2:** 57–62.

Ockelmann, K. W. 1964. *Turtonia minuta* (Fabricus), a neotenous veneracean bivalve. *Ophelia* **1:** 121–146.

Padilla, M., M. Mendez, and F. Casanova. 1969. Observaciones sobre el comportamiento de la *Ostrea chilensis* en Apiao. *Bol. Inst. Fomento Pesquero, Santiago de Chile.* **10:** 1–28.

Pechenik, J. A. 1979. Role of encapsulation in invertebrate life histories. *Am. Nat.* **114:** 859–870.

Pechenik, J. A. 1986. The encapsulation of eggs and embryos by molluscs: an overview. *Am. Malacol. Bull.* **4:** 165–172.

Solís, I. 1967. Observaciones biológicas en ostras (*Ostrea chilensis* Philippi) de Pullinque. *Biol. Pesq.* **2:** 51–82.

Solís, I. 1973. Valoración de colectores de larvas de ostras *Ostrea chilensis* Philippi en Pullinque. *Biol. Pesq.* **6:** 1–23.

Strathmann, R. R. 1978. The evolution and loss of feeding larval stages in marine invertebrates. *Evolution* **32:** 894–906.

Strathmann, R. R., and E. Leise. 1979. On feeding mechanisms and clearance rates of molluscan veligers. *Biol. Bull.* **157:** 524–535.

Strathmann, R. R., T. L. Jahn, and J. R. C. Fonseca. 1972. Suspension feeding by marine invertebrate larvae: clearance of particles by ciliated bands of a rotifer, pluteus, and trochophore. *Biol. Bull.* **142:** 505–519.

Tankersley, R. A., and R. V. Dimock. 1992. Quantitative analysis of the structure and function of the marsupial gills of the freshwater mussel *Anodonta cataracta. Biol. Bull.* **182:** 145–154.

Tankersley, R. A., and R. V. Dimock. 1993. Endoscopic visualization of the functional morphology of the ctenidia of the unionid mussel *Pyganodon cataracta. Can. J. Zool.* **71:** 811–819.

Toro, J. E., and O. R. Chaparro. 1990. Conocimiento biológico de *Ostrea chilensis* Philippi 1845. Impacto y perspectivas en el desarrollo de la ostricultura en Chile. Pp. 231–264 in *Cultivo de Moluscos en América Latina. Memorias Segunda Reunión Grupo Trabajo Técnico, Ancud, Chile. Noviembre 1989.* A. Hernández, ed. Special publication, Canadian International Development Agency, Bogotá, Colombia.

Waller, T. R. 1981. Functional morphology and development of veliger larvae of the European oyster, *Ostrea edulis* Linné. *Smithson. Contrib. Zool.* **328:** 1–70.

Winter, J. E., J. E. Toro, J. M. Navarro, G. S. Valenzuela, and O. R. Chaparro. 1984. Recent developments, status, and prospects of molluscan aquaculture on the Pacific coast of South America. *Aquaculture* **39:** 95–134.

Yonge, C. M. 1926. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis. J. Mar. Biol. Assoc. U. K.* **14:** 295–386.